FBS01 – General Procedures for Forensic Biological Evidence Examination

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1. Scope

1.1. Analysts will follow the procedures listed below when analyzing evidentiary items for the presence of biological fluids and/or DNA.

2. Background

2.1. This procedure is used to establish general practices for the examination of biological evidence and for documenting the examination of biological evidence.

3. Safety

3.1. Wear personal protective equipment (PPE) (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

4. Materials Required

4.1. Not applicable

5. Standards and Controls

5.1. All sample collection procedures must be performed in dedicated laboratory space to maintain their separation from all sources of amplified DNA product.

5.2. When using pipettes as part of sample collection, pipettes dedicated to pre-PCR amplification set-up activities must be used.

5.3. A separation in time and space must be maintained between questioned and known samples during inventory, examinations, cutting, and re-packaging.

5.4. To maintain separation in time and space between individual samples, each sample collected must be placed in a sample tube, and that tube closed, before any other sample from that item can be collected. If no other sample is to be taken from an item, then that item must be repackaged before the next item may be opened and processed for sample collection.

5.5. Only one case will be examined at a time and only one sample will be opened and processed at any one time.

5.6. Casework notes must be recorded contemporaneously with each procedure.

6. Procedures

6.1. Appropriate measures will be taken throughout the testing process to avoid contamination. The precautions listed below will be followed during each procedure to ensure the quality of work and accuracy of results:

6.1.1. All work surfaces are thoroughly cleaned with 10% bleach followed by 70% ethanol prior to and after the examination of each separate item in a case. This practice can also be performed more often if examining a heavily soiled item of evidence.

6.1.2. Disposable bench paper is used to prevent the accumulation of biological material on permanent work surfaces. At a minimum, the paper is changed between items of evidence or more frequently if examining a heavily soiled item of evidence. Disposable bench paper is discarded in appropriate containers. Disposable bench paper will be placed in biohazard trash only when visibly soiled with other potentially infectious materials (OPIM).

6.1.3. Wear appropriate PPE (e.g., lab coat, gloves, masks, eye protection, hair net). Change gloves or clean gloves with 10% bleach and 70% ethanol between items of evidence or more frequently when visibly

soiled. Gloves are discarded in biohazard containers if visibly soiled. Hands will be thoroughly washed when leaving laboratory space.

6.1.4. Lab coats are worn at all times. A dedicated laboratory coat must be worn for all pre-amplification sample handling activities. A separate, dedicated laboratory coat must be worn when handling samples that may potentially contain amplified DNA.

6.1.5. Writing instruments (e.g., pens, markers) are thoroughly cleaned with 10% bleach followed by 70% ethanol prior to and after the examination of each item in a case.

6.1.6. Metal tools (e.g., forceps, scissors) used during examinations are thoroughly cleaned with 10% bleach followed by 70% ethanol before and after coming in contact with an item of evidence. Metal tools may also be autoclaved to sterilize. After use, disposable utensils are discarded in the appropriate trash.

6.1.7. Any evidence of considerable size (e.g., bed sheets, comforters) will be examined in a size appropriate space. Disposable paper may be placed on the floor under such items if they are suspended for examination.

6.1.8. All evidence items under active examination are analyzed in distinct workspaces away from other items of evidence under examination by other individual(s) working within a common laboratory space.

6.1.9. To prevent indirect transfer of biological material to telephones and vestibule door handles, clean disposable gloves must be worn to handle such laboratory equipment.

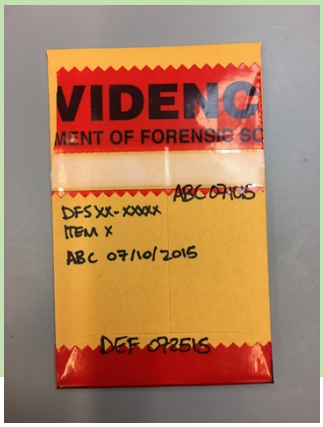
6.1.10. During a common procedure step, sample tubes must remain closed unless being processed. Only one sample or reagent tube can be open at a given time during the processing of the individual samples of a case or batch.

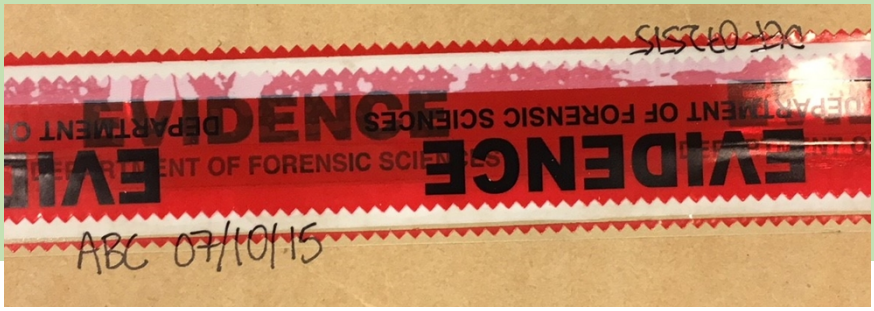
6.1.11. Prior to leaving the laboratory vestibule area and entering non-laboratory areas, always remove all PPE, dispose of gloves and wash hands.

6.2. Prior to the analysis of evidentiary material, when practicable, the relevant elements of each case will be evaluated through communication (e.g., police reports, medical reports, discussions with investigators) with the submitting agency and/or attorneys. This evaluation will include an assessment of the evidence and its relevance. Based on this information, the items to be tested for the case will be documented in the JusticeTrax Laboratory Information Management System (JT LIMS).

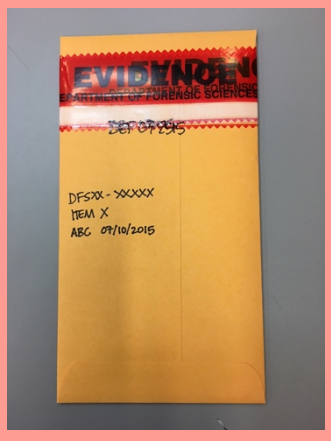
6.3. Whenever possible, analysts will open a container by avoiding a previous seal. In cases where this is not possible (i.e., boxed items or items which have been previously opened several times), analysts will ensure that previous seals are able to be seen when re-sealing the container. Analysts will not add their analyst markings over previous analyst markings.

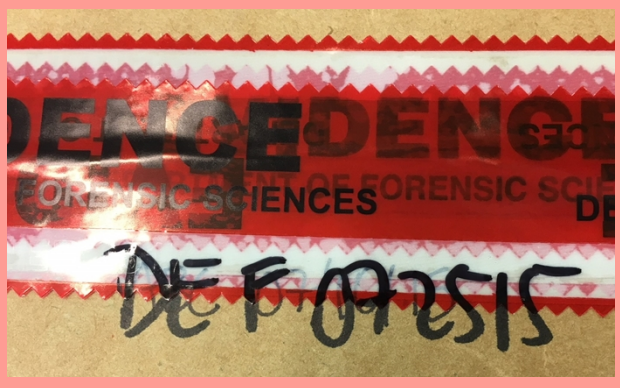
Examples of **CORRECT** re-sealing of evidence:





Examples of **INCORRECT** re-sealing of evidence:





6.4. Each evidence item will be examined separately on a clean work surface. The examination of each evidence item will include an assessment for all potential physical evidence (e.g., physiological fluid stains, hairs, fibers, gunshot residue, latent prints).

6.5. If trace evidence (e.g. hairs, fibers) is encountered during an examination, the evidence will be collected, if possible, prior to any further examinations, labeled

to indicate what it is and where it was located, properly packaged to prevent loss, and a chain of custody will be initiated in JT LIMS.

6.6. The FBU will only make gross observations regarding evidence suspected of being hairs or fibers. Analysts will refer to this type of evidence as "possible hairs" or "possible fibers" in case notes and reports. Characteristics such as color, length, convolution and presence/absence of adhering material may be noted. Detailed characterization (e.g., stage of root growth, species of origin, condition, fiber type) and comparisons will only be made by qualified trace evidence analysts.

6.7. Ideally evidence items will be processed for latent prints prior to submission to the FBU for examination. If an item is to be examined by the FBU prior to latent print processing, the analyst will take all necessary precautions to ensure any latent prints are not compromised during the examination (i.e., wear cotton gloves under disposable gloves).

6.8. Detailed information including description of evidence packaging and seal(s) as received (refer to *DOM10- Procedures for Handling Evidence and Clinical Specimens* (Document Control Number: 1281) and *LOM01- Procedures for the Examination of Evidence* (Document Control Number: 1315) for proper seal guidelines), item description, size, color, condition, visible stains, etc., will be recorded in the applicable JT LIMS documentation. In addition, record location of apparent stab or bullet holes and/or other obvious damage. All evidentiary stains on the item must also be documented to note the location, size, color, condition, visibility, etc.

6.8.1. Photographs and/or diagrams may also be included to document the condition of the evidence prior to, or during examination(s). If taken, the photograph will contain, at a minimum, the item being examined, a scale, date and initials of the examiner, DFS case #, and DFS item #. Photographs will be uploaded under the appropriate evidence number in the imaging module of JT LIMS.

6.8.2. Record in JT LIMS if a sample/stain is consumed.

6.9. Evaluate each stain in the following systematic manner and record observations on the appropriate JT LIMS and/or Sample Tracking and Control Solutions (STACS) documentation. (Note: An analyst may evaluate and test representative stains; however, the evidence exam notes must clearly state that representative stains were taken). Depending on the information obtained from the submitter, not all questions will be addressed. For items not requiring biological fluid examination(s) skip to section 6.9.4.

6.9.1. Is the stain blood?

6.9.1.1. This question is answered by visual and chemical testing. Normally, bloodstains are fairly easy to locate due to their distinctive red/brown color. However, if they occur on a dark colored background, are faint or small in size, they may prove to be difficult to locate without the use of careful searching techniques and specialized lighting. The shape and size of the stain can be important evidence and will be documented (e.g., notes, diagrams, photography) prior to actual sampling, which might alter the interpretive value of the stain pattern. The information recorded will include location, size, color, and may contain additional information such as: shape, concentration (e.g. diluted), and/or stain type (e.g., smear, transfer, droplets).

6.9.2. Does the stain contain semen or seminal fluid?

6.9.2.1. This question is answered by visual, microscopic, chemical and/or immunological testing. Potential semen stains can be visually located with the use of careful searching techniques and specialized lighting (*FBS04 – Use of Alternate Light Source to Aid in Stain Identification* (Document Control Number: 1573)).

6.9.3. Is the stain a mixture of body fluids?

6.9.3.1. This question can be answered by performing a panel of chemical, microscopic and immunological tests.

6.9.4. Which samples will be collected for DNA testing?

6.9.4.1. Based on the provided case information, the analyst will evaluate the evidence and determine which samples may have probative value. A sampling of any probative sample may be collected for DNA analysis and placed into an appropriately labeled, sterile microcentrifuge tube. If not immediately processing sample, store in the appropriate location (e.g., room temperature, refrigerator, freezer). The size of the sample collected will depend on various factors including the stain concentration, size, and/or the results of presumptive and confirmatory testing. Every effort will be made to retain a portion of the evidence, either in its original form or as an extract.

6.9.4.1.1. Refer to *LOM01 – Practices for the Examination of Evidence* (Document Control Number: 1315), Section 5.4 for evidence

subdividing procedure for creation of unique identifying numbers for stain/swab cuttings to be taken forward for DNA analysis.

6.9.4.1.2. Available reference samples will also be collected for typing and comparison. These samples are typically received as liquid blood, bloodstains, saliva stains or buccal swabs.

6.9.4.1.2.1. If received as liquid blood, a portion will be spotted onto a Whatman FTA® card and dried for preservation purposes (*FBS27 – Preparation of FTA Bloodstain Card from Liquid Blood* (Document Control Number: 4276)) and documented in the applicable JT LIMS documentation.

6.9.4.2. The sampling method used will depend on various factors such as type of suspected biological material and type of substrate (porous vs. non-porous). Examples of sampling methods include:

6.9.4.2.1. Cutting: Utilize a sterile scalpel blade or sterilized scissors to remove the relevant portion of the item/stain.

6.9.4.2.2. Wet/dry swabbing: Wet one sterile cotton swab with autoclaved deionized water. Record the lot number of the water in the JT LIMS notes section for the relevant item. Swab the area of interest with the wet swab followed by a dry swab. Rotate each swab as you sample.

6.9.4.2.3. Scraping: Utilize a sterile scalpel blade, hold at ~45° angle, and scrape the area of interest to generate small scrapings or pieces of the material. Collect these scrapings or pieces for testing.

6.9.4.3. The following are general guidelines for sampling items for extraction. Case information, direct observation of the item and analyst experience may further dictate sampling.

6.9.4.3.1. Known buccal swabs: Cut ~1/4 of swab.

6.9.4.3.2. Known blood cards: Cut ~0.3cm x 0.3cm from stain.

6.9.4.3.3. Swab for touch DNA: Cut the outermost portion of swab. The biological material from the swab will be considered consumed in this instance. If the swab was submitted to the FBU, the swab stick will be retained and repackaged as received. If the swab was created by the FBU (i.e., swabbing a rope for touch DNA), it is FBU's practice to discard the swab stick.

6.9.4.3.4. Swabs from sexual assault kits (non-touch): For samples suspected of containing semen, target ~1 swab's worth of material. For example, if a swab box labeled "anal swabs" contains 3 swabs, cut ~1/3 of each swab and combine all the cuttings into one sample. If one swab box is labeled "vaginal swabs" and one swab box is labeled "cervical swabs" these should be treated as two separate samples. For samples not suspected of containing semen (i.e., fingernail scrapings), test as outlined in section 6.9.4.3.3.

6.9.4.3.5. Cigarette butts: Cut ~1.0cm x 1.0cm from filter paper on mouth end.

6.9.4.3.6. Suspected semen or blood stains: Cut ~0.5cm x 0.5cm from stain. A larger or smaller cutting may be taken depending on the appearance of the stain (i.e. faint vs dark) and results of serological testing (if performed).

6.9.5. Does the sample contain human DNA?

6.9.5.1. It may be inferred that the biological material is of human origin through the use of a human specific PCR test (e.g., Plexor® HY kit, PCR Amplification Kits).

6.9.6. When samples are to be outsourced or further downstream analyses are to be conducted by different FBU analyst(s), the appropriate casework documentation (e.g., STACS documentation, data results print-outs,

electropherograms, examination report(s)) will be submitted to the outsource lab or assigned FBU analyst(s) accompanying the samples.

6.9.7. Who could have contributed the stain(s)?

6.9.7.1. This question is answered through the use of DNA typing systems. The cutting or swabbing is taken through a series of temperature and chemical processes in order to extract the DNA from the cells, assess the quantity of the DNA obtained, generate multiple copies of specific areas of the DNA and finally establish the DNA profile(s) of the contributor(s) of the original biological material. The evidence DNA profile(s) are then evaluated to determine if they are suitable for interpretation. If they are suitable for interpretation, the profile(s) may be uploaded to CODIS and/or compared to known/reference DNA profiles to determine if an individual of interest is included or excluded as a possible source of the genetic material.

6.9.8. What is the significance of an inclusion?

6.9.8.1. In 2000 the DNA Advisory Board (DAB) stated, “When a comparison of DNA profiles derived from evidence and reference samples fails to exclude an individual(s) as a contributor(s) of the evidence sample, statistical assessment and/or probabilistic reasoning are used to evaluate the significance of the association”. SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories (2017) state, “Except for a reasonably assumed contributor, the laboratory shall perform statistical analysis in support of any inclusion (or a “cannot be excluded” conclusion), irrespective of the number of alleles detected and the quantitative value of the statistical analysis.”

6.9.8.2. Statistical interpretation attempts to provide meaning/weight to the findings. The significance of an inclusion is expressed in terms of a likelihood ratio (LR). A likelihood ratio is a ratio of the probability of obtaining the evidence (DNA profile) given competing propositions/hypotheses.

$$LR = \frac{\text{Proposition of prosecution (Hp)}}{\text{Proposition of defense (Hd)}}$$

6.9.8.3. For capillary electrophoresis (CE) testing, the likelihood ratio is calculated using allele frequencies referenced from the 2015 FBI Expanded Loci population data set and the sub-population model developed by Balding and Nichols in 1994. For information about calculating likelihood ratios from samples tested with Next Generation Sequencing (NGS), refer to the ForenSeq™ DNA Interpretation Guidelines.

6.10. The reliability of a particular test or analysis is demonstrated through the use of appropriate methods, controls, standards, blanks and proficiency tests.

6.11. Further evaluation of samples may be warranted when unexpected results and/or results that cannot be interpreted or compared occur, such as:

6.11.1. Re-evaluation of the sample selection (e.g. cutting, swabbing or scraping of stain/item)

6.11.2. Evaluation of sample age and possible substrate interferences

6.11.3. Re-analysis using the same conditions.

6.11.4. Re-analysis using different conditions or alternative methods.

6.11.5. Recognition of ambiguous results; report as “uninterpretable”

6.11.6. Refer to supervisor for direction

6.11.7. Independent re-analysis by another qualified analyst

6.11.8. Evaluation of the amount of remaining evidence and value of re-analysis

6.12. Casework notes are the basis for the written report. Reports are prepared in accordance with laboratory policy. Refer to *LOM02 – Procedures for Case Documentation and Report Writing* (Document Control Number: 1319).

6.12.1. Casework notes will be made contemporaneously to the testing and examinations, and must reflect analytical results and observations. Notes are permanent records that reflect evidence conditions, techniques and methodology used, data and conclusions.

6.12.2. Casework notes are intended to: (a) refresh the analyst’s memory; (b) document the approach, observations, methodology, results and conclusions; and (c) allow interpretations to be made.

6.13. Any deviation from laboratory protocol must first be approved by the DNA Technical Leader or designee and must be documented in the case notes.

Otherwise, it may be assumed that the analysis proceeded according to the laboratory policies and procedures.

6.13.1. Refer to *DOM17 – Procedures for Authorizing Deviations* (Document Control Number: 4037) for procedure regarding deviations.

6.14. The final step in the process is to write a report which reflects all of the testing results and interpretations from the case. The entire case file and report are then technically and administratively reviewed to verify that all conclusions are appropriate and supported by documentation.

6.15. If there is a conflict, uncertainty or dispute between the conclusions of the analyst and reviewer, the case will be discussed and reviewed with the DNA Technical Leader. If necessary, the final conclusion of the DNA Technical Leader will stand as the official conclusion for the report and must be adhered to by the original analyst.

6.15.1. The discussion and resolution of the conflict, uncertainty or dispute will be documented in the case file's JT LIMS Case Activity Log.

6.16. At the completion of testing, DNA extracts and associated reagent blanks will be properly packaged to prevent loss, and a chain of custody will be initiated in JT LIMS.

7. Sampling

7.1. Not applicable

8. Calculations

8.1. Not applicable

9. Uncertainty of Measurement

9.1. Not applicable

10. Limitations

10.1. Not applicable

11. Documentation

11.1. Applicable STACS documentation

11.2. Applicable JT LIMS documentation

11.3. FBU Report of Examination

12. References

12.1. Balding, D.J. and R.A Nichols, DNA profile match probability calculation: how to allow for population stratification, relatedness, database selection and single bands. Forensic Science International, (1994). 64: 125-140.

12.2. SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, (2017).

12.3. DNA Advisory Board. (2000). Statistical and population genetic issues affecting the evaluation of the frequency of occurrence of DNA profiles calculated from pertinent population database(s). Forensic Sci. Comm, 2(3).

12.4. Forensic Biology Unit Quality Assurance Manual

12.5. Use of Alternate Light Source to Aid in Stain Identification (FBS04)

12.6. Preparation of FTA Bloodstain Card from Liquid Blood (FBS27)

12.7. Procedures for the Examination of Evidence (LOM01)

12.8. Procedures for Case Documentation and Report Writing (LOM02)

12.9. Procedures for Handling Evidence and Clinical Specimens (DOM10)

12.10. Procedures for Authorizing Deviations (DOM17)